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2.5. Cell proliferation assay

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An abbreviated version of this protocol was published in Molecular Oncology in Jan 2019

Circular RNA profiling identifies circADAMTS13 as a miR-484 sponge which suppresses cell proliferation in hepatocellular carcinoma

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Detailed protocol

Cell proliferation assay protocol

Cell proliferation assays were carried out using the cell counting kit-8 (CCK-8) assay and colony formation assay.

CCK-8 assay

Stably overexpressing circADAMTS13 cells and corresponding wild-type cells were resuscitated and incubated at 37 °C in 5% CO_2.

Day1

1. Cells were cultured to the logarithmic growth phase, then cells were digested with trypsin and the concentration of cells was adjusted to 5×10^4 /ml.
2. Five 96-well plates were taken to correspond to 8h, 24h, 48h, 72h and 96h time groups. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well and incubated at 37 °C in 5% CO2. Each cell had 5 replicates at each time point.

Day1~5

Further experiments were performed at each time point.

1. The test solution was prepared according to the ratio of medium: CCK-8 solution = 9:1(According to the instructions of the CCK-8 reagent kit).
2. Absorb and discard the supernatant, 100 μl per well test solution was added into the 96-well plate.
3. After incubation for 2h in an incubator at 37℃ and 5%CO_2.
4. The absorbance was measured at the wavelength of 450 nm (A450) according to the manufacturer's protocol.
5. Calculate the Relative A450 of cells at each time point according to the following formula:

Relative A450[x h] = (A450[x h]-A450[8 h])/ A450[8 h]

Colony formation assay

Stably overexpressing circADAMTS13 cells and corresponding wild-type cells were resuscitated and incubated at 37 °C in 5% CO_2.

1. Cells were cultured to the logarithmic growth phase, then cells were digested with trypsin and the concentration of cells was adjusted to 5×10^4 /ml. Each experimental group had 3 duplicate holes.
2. Cells were seeded in 6-well plates at a density of 5×10^2 cells per well and incubated at 37 °C in 5% CO_2 for 2~3 weeks. The cell culture medium was changed every 3 days.
3. The staining was performed when cell clone formation was observed. The colonies were fixed with 4% paraformaldehyde for 20 min and then stained with 0.1% crystal violet for 10 min.
4. Wash cell colonies with ddH_2O for several times until the background color faded and the cell cloning boundaries were clear, dried.
5. Wells were photographed with a gel imager. The specific number of cell clones were counted by Image J software.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Cai, Z. , Liu, X. and Liu, J. (2021). 2.5. Cell proliferation assay. Bio-protocol Preprint. bio-protocol.org/prep1229.
2. Qiu, L., Huang, Y., Li, Z., Dong, X., Chen, G., Xu, H., Zeng, Y., Cai, Z., Liu, X. and Liu, J.(2019). Circular RNA profiling identifies circADAMTS13 as a miR-484 sponge which suppresses cell proliferation in hepatocellular carcinoma. Molecular Oncology 13(2). DOI: 10.1002/1878-0261.12424